

Patent claims.

CLAIMS

1. (Original) A method for the production of a lysate used for cell-free protein biosynthesis, comprising the following steps:

a) a genomic sequence in an organism, which codes for an essential translation product that reduces the yield of cell-free protein biosynthesis, is replaced by a foreign DNA located under a suitable regulatory element, said foreign DNA coding for the essential translation product that additionally contains a marker sequence;

b) the transformed organism according to step a) is cultivated;

c) the organisms from the culture obtained in step b) are lysed; and

d) the essential translation product is separated from the lysate obtained in step c) by means of a separation process that is selective for the marker sequence.

2. (Currently Amended) A method according to claim 1, ~~characterized by that wherein~~ the essential translation product is selected from the group consisting of [["]]]termination factors or proteins interacting with termination factors - in particular RF1, RF2, RF3, eRF, L11 or HemK -, initiation factors or proteins interacting with initiation factors, elongation factors or proteins interacting with elongation factors, aminoacyl tRNA synthetases - in particular cysteinyl tRNA or tryptophanyl tRNA synthetase -, enzymes of the amino acid metabolism - in particular amino acid transferases, isomerases, synthetases -, phosphatases, nucleases, proteases, kinases, racemases, isomerases, polymerases and combinations of the above substances["]].

3. (Currently Amended) A method according to ~~one of claims 1 or 2, characterized by that claim 1, wherein~~ the marker sequence is selected from the group consisting of ["]streptag II, polyhistidine, FLAG, polyarginine, polyaspartate, polyglutamine, polyphenylalanine, polycysteine, Myc, glutathione S-transferase, protein A, maltose-binding protein, galactose-binding protein, chloramphenicol acetyl transferase, protein G, calmodulin, calmodulin-binding peptide, HAT (= natural histidine affinity tag), SBP (= streptavidin-binding peptide), chitin-binding domain, thioredoxin, β -galactosidase, S-peptide (residues 1-20 of the Rnase A), avidin, streptavidin, streptag-I, dihydrofolate reductase, lac repressor, cyclomaltodextrin glucanotransferase, cellulose-binding domain, btag, nanotag["].

4. (Currently Amended) A method according to ~~one of claims 1 to 3~~ claim 1, wherein the marker sequence and the chromosomal gene are expressed as a fusion protein, and wherein the translated marker sequence does not affect the activity of the essential translation product in the organism.

5. (Currently Amended) A method according to ~~one of claims 1 to 4~~ claim 1, wherein the separation step is an affinity chromatography or an antibody assay.

6. (Currently Amended) A method according to ~~one of claims 1 to 5, characterized by that claim 1, wherein~~ the organism is a prokaryote or an eukaryote, in particular selected from the group comprising ["]enterobacteriales (e.g. escherichia spec., E. coli), lactobacillales (e.g. lactococcus spec., streptococcus spec.).

actinomycetales (e.g. streptomyces spec., corynebacterium spec.), pseudomonas spec., caulobacter spec., clostridium spec., bacillus spec., thermotoga spec., micrococcus spec., thermus spec.[["]].

7. (Currently Amended) A lysate for the cell-free protein biosynthesis obtainable by a method according to ~~one of claims 1 to 6, claim 1,~~ wherein the lysate has a reduced activity of an essential translation product.

8. (Currently amended) A lysate for the cell-free protein biosynthesis according to claim 7, wherein the lysate has a reduced activity of one or several essential translation products selected from the group consisting of ["]]termination factors or proteins interacting with termination factors - in particular RF1, RF2, RF3, eRF, L11 or HemK -, initiation factors or proteins interacting with initiation factors, elongation factors or proteins interacting with elongation factors, aminoacyl tRNA synthetases - in particular cysteinyl tRNA or tryptophanyl tRNA synthetase -, enzymes of the amino acid metabolism - in particular amino acid transferases, isomerases, synthetases -, phosphatases, nucleases, proteases, kinases, racemases, isomerases, polymerases and combinations of the above substances[["]].

9. (Currently Amended) The use of a lysate according to claim 7 ~~or 8~~ for the cell-free protein biosynthesis.

10. (Currently amended) The use according to claim 9, wherein by means of amber suppressor tRNA's natural and/or non-

natural amino acids, in particular biotinyl-lysine, fluorescent amino acids and/or phenyl-analine, are incorporated.

11. (Original) An isolated microorganism or an isolated cell, wherein a genomic sequence, which codes for an essential translation product that reduces the yield of cell-free protein biosynthesis is replaced by a foreign DNA located under a suitable regulatory element, said foreign DNA coding for the essential translation product that additionally contains a marker sequence.

12. (Original) A microorganism, as deposited under DSM 15756.